ENZYME HISTOCHEMISTRY OF ACUTE STAPHYLOCOCCAL ENTEROTOXIN GASTROENTERITIS IN RHESUS MONKEYS

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Sequential changes in mucosal enzyme activity in various forms of gastroenteritis have not been extensively studied. Recovery of histochemically demonstrated enzyme activity has been investigated in humans with celiac disease (non-tropical sprue) after institution of a gluten-free diet,[1,2] and sequential changes in enzyme activity in the small intestinal mucosa of mice have been studied after whole-body irradiation.[3,4] Additional diseases of the gastric or small intestinal mucosa examined by enzyme histochemical methods include chronic gastritis,[5,6] Whipple's disease [1,7] and cholera [8] in humans, a protozoan infection in guinea pigs [9] and a nematode infection in rats.[10]

The development of a model for the production of acute gastroenteritis in rhesus monkeys with staphylococcal enterotoxin [11] afforded an opportunity to correlate changes in mucosal enzyme activity with the development and regression of the lesion. After intragastric administration of enterotoxin rhesus monkeys develop an acute gastroenteritis which is well developed by 2 hours, maximal at 4 to 8 hours, and gradually regresses from 12 to 72 hours. In the gastric mucosa the principal reacting cells are leukocytes and mucous cells. In the jejunum the early leukocytic reaction is followed by degenerative changes in epithelial cells, elongation of crypts and shortening of villi. In addition to the enzyme histochemical changes, the alterations in mucosal lipid content will also be correlated with the morphologic features. Since there is little information on the distribution of enzyme activity in the gastrointestinal tract of rhesus monkeys, observations in normal animals will be described.

MATERIAL AND METHODS

Rhesus monkeys (*Macaca mulatta*) weighing 2 to 3 kg were given 150 μg purified staphylococcal enterotoxin B in 20 ml of 0.9 per cent NaCl by gastric tube. The principl s of laboratory animal care as promulgated by the National Society for Medical Research were observed. Groups of 3 animals were killed at 2, 4, 8, 12, 24, 48 and 72 hours after administration of enterotoxin. Five untreated animals were

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killed at suitable times to serve as controls for enzyme reactions. Tissue was taken
from 4 sites: greater curvature of the stomach including fundus and body, greater
curvature including antrum and body, proximal jejunum, and distal ileum. Strips
of tissue were rolled, quenched in isopentane cooled by liquid nitrogen and stored
in air-tight jars in a dry ice chest.

Blocks were sectioned within 24 hours in a cryostat at 6 to 8 μ. Air dried sections,
fixed in cold acetone except in glucose-6-phosphatase study, were incubated with the
appropriate substrates. The enzymes or enzyme systems investigated were as fol-
lows: 1) alkaline phosphatase using naphthol AS-MX phosphate as substrate and
fast red violet LB salt [12]; 2) acid phosphatase using naphthol AS-TR phosphate as
substrate and diazotized pararosanilin [13]; 3) glucose-6-phosphatase [14]; 4) reduced
diphosphopyridine nucleotide (DPNH) diaphorase [13]; 5) reduced triphosphopyri-
dine nucleotide (TPNH) diaphorase [13]; 6) succinic dehydrogenase [13]; 7) glucose-6-
phosphate dehydrogenase [15]; and, 8) hexokinase system.

The hexokinase technique developed by Kuhns (in preparation), demonstrates
the glucose phosphorylating enzyme using glucose as substrate and exogenous
glucose-6-phosphate dehydrogenase and endogenous TPNH diaphorase to reduce the
tetrazolium salt. The substrate solution contains the following: glucose, 50 mg;
TPN (monosodium salt), 1.4 mg; ATP (disodium salt), 5.5 mg; Mg Cl_2, 30 mg;
NBT, 2.5 mg; 0.02M phosphate buffer, 2 ml; distilled water, 4 ml; glucose-6-
phosphate dehydrogenase (Sigma Chemical Co., St. Louis, Mo.), 10 units. The final
volume was 6 ml at pH 7.5. Incubation time was 1 hour at 37° C.

Appropriate controls were run to establish the validity of all enzyme reactions.
Parallel frozen sections were fixed in buffered 10 per cent formalin and stained with
oil red O for the demonstration of lipid. General morphologic characteristics were
evaluated in formalin fixed tissue embedded in paraffin and stained with hematoxylin
and eosin.

RESULTS

Stomach

The morphologic changes in the gastric mucosa of rhesus monkeys
following intragastric enterotoxin have been described in an earlier re-
port.[11] In general, the lesion involved predominantly the fundic and
antral mucosa. Only focal superficial leukocytic infiltrate occurred in
the body mucosa. The lesion was maximal at 4 to 8 hours. It was char-
acterized by leukocytic infiltration and distention of mucus secretory
cells followed by their depletion. No changes were observed in parietal
cells, but chief cell cytoplasm appeared shrunken at the height of the
lesion.

The enzyme histochemical changes in the gastric mucosa were not
striking. There was a slight decrease in activity of several enzymes in
the surface mucous cells at the height of the lesion and a slight increase
in acid phosphatase activity in chief cells. A more detailed description
of the changes in enzyme activities follows.

Phosphatases. In control and experimental animals strong alkaline
phosphatase activity was present only in arterioles and smooth muscle
fibers of the gastric mucosa. In control animals acid phosphatase ac-
tivity was moderate in the cytoplasm of chief cells, weak to absent in

parietal cells, moderate in the supranuclear zone of surface mucous cells, moderate and diffuse in the cytoplasm of deep pyloric mucous cells, and strong in widely scattered macrophages. In animals with acute gastritis there was a moderate increase in the activity in the cytoplasm of chief cells (Figs. 1a and 1b). The surface mucous cells had a slight decrease in activity at the height of the lesion, but later activity appeared slightly increased in foveolar and surface cells. At the height of the lesion the mucosa was infiltrated with many macrophages exhibiting strong acid phosphatase activity. No significant glucose-6-phosphatase activity was demonstrated in the stomach.

Oxidative Enzymes (DPNH diaphorase, TPNH diaphorase, succinic dehydrogenase and glucose-6-phosphate dehydrogenase). In control animals these enzymes exhibited strong activity in parietal cells. Surface mucous cells had strong glucose-6-phosphate dehydrogenase and TPNH diaphorase activity, moderate activity of succinic dehydrogenase and weak DPNH diaphorase activity. Activity of these enzymes was slight in foveolar mucous cells and absent in deep mucous glands and chief cells. In animals with acute gastritis enzyme activity was slightly decreased in surface mucous cells but unchanged in parietal cells.

Hexokinase System. In control animals the surface mucous cells exhibited strong activity; it was moderate in parietal cells. There was a mild decrease in activity in surface cells at the height of the lesion when the epithelium was depleted of mucus.

Small Intestine

At 2 hours the jejunal mucosa was characterized by leukocytic infiltration, focal epithelial degeneration at the tips of villi, and slight crypt lengthening; at 4 to 8 hours there were diffuse epithelial degeneration and sloughing, villus shortening, crypt lengthening, and decreasing leukocytic infiltrate; from 12 to 72 hours rapid maturation of surface epithelium was apparent and there were progressive increase in villus height, shortening of crypts and decreasing leukocytic infiltration.

In the jejunal mucosa some of the enzymes studied exhibited a mild decrease in activity at 2 hours after enterotoxin administration. At 4 and 8 hours, activity of all the enzymes studied was severely decreased. Enzymatic activity returned to near control levels by 24 hours and was usually indistinguishable from normal at 48 to 72 hours. The most severe decrease in enzyme activity corresponded with the most severe epithelial degeneration which was seen at 4 and 8 hours. The enzyme decrease was most pronounced on the surface and upper portions of the villi.

The ileum exhibited a much less severe reaction with only slight villus

epithelial and crypt changes. Slight decreases in enzyme activity occurred at 4 and 8 hours.

The following description of the distribution of enzyme activity in control and experimental monkeys applies to the jejunal mucosa.

Alkaline Phosphatase. In control animals strong activity which faded toward the base of villi appeared in the brush borders (Fig. 2a). Weak activity was present in the Golgi zone of villus epithelium and in the muscularis mucosae. Arterioles exhibited strong activity. In experimental animals there was a slight decrease in activity in the brush border at 2 hours and severe decrease at 4 hours. At 8 and 12 hours there was practically no activity on the surface of epithelial cells (Fig. 2b). By 24 hours the activity returned to near normal (Fig. 2c). At the height of the lesion activity in the Golgi zone disappeared but no change was noted in the other sites.

Acid Phosphatase. In control animals the apical cytoplasm and Golgi zone of villus epithelium exhibited distinct activity (Fig. 3a). Strong activity was also present in Paneth cells and macrophages. Patchy activity in the brush border was not inhibited by 0.01M NaF. Activity not inhibited by fluoride is not considered to be attributable to true acid phosphatase.[16] In experimental animals apical activity was moderately decreased at 2 hours, severely decreased at 4 and 8 hours (Fig. 3b) and gradually returned to normal by 48 hours. Activity in the Golgi zone was slightly decreased at 4 and 8 hours. There was no change in Paneth cell activity. The number of macrophages increased strikingly at the peak of the lesion. At 4 hours macrophages had strong acid phosphatase activity, but at 8 hours many had less than comparable cells in control animals (Figs. 3a and 3b).

Glucose-6-phosphatase. In controls activity was present in villus epithelial cytoplasm (Fig. 4a). In experimental animals activity was moderately decreased at 4 to 12 hours (Fig. 4b) and gradually returned to normal by 72 hours.

Oxidative Enzymes (DPNH diaphorase, TPNH diaphorase, succinic dehydrogenase, glucose-6-phosphate dehydrogenase). In controls activity of these enzymes was strongest in villus epithelial cytoplasm (Figs. 5a, 6a and 7a). Succinic dehydrogenase activity was stronger in the basal part of the cells and the other enzymes had stronger activity in the apical zone. The basal area of crypts had moderate activity only for DPNH diaphorase and succinic dehydrogenase and the upper part of the crypt had moderate activity only for DPNH diaphorase. In experimental animals villus epithelium exhibited a slight decrease in activity at 2 hours, a moderate to severe decrease at 4 and 8 hours (Figs. 5b, 6b and 7b), improvement at 12 hours and a return to near normal by 24 hours (Figs. 5c, 6c and 7c).

Hexokinase System. In control and experimental animals activity demonstrated by this method had a similar distribution to that of TPNH diaphorase and glucose-6-phosphate dehydrogenase (Figs. 8a, 8b and 8c).

Mucosal Lipid. In the jejunum but not in the ileum of controls fine oil red O stained droplets were evident in the epithelium and lamina propria of villi (Fig. 9a). In experimental animals there was a striking change in mucosal lipid distribution. There was a severe decrease or absence of oil red O staining droplets in the mucosa of the jejunum from 2 to 8 hours after enterotoxin administration but by 12 hours lipid was again prominent (Figs. 9b and 9c). In the ileum lipid droplets were prominent in the epithelium and lamina propria from 4 to 12 hours but disappeared by 24 hours.

DISCUSSION

A significant finding in this study was the close correlation between degeneration of epithelium in the small intestine and a decrease in enzyme activity of these cells. It has also been demonstrated that small intestinal mucosal enzyme activity decreased and recovered rapidly following acute injury. In the jejunum the activity of all the enzymes studied was depressed; the activity of oxidative enzymes (DPNH and TPNH diaphorase, succinic dehydrogenase, glucose-6-phosphate dehydrogenase) began, however, to decrease sooner (2 hours) and returned toward normal earlier (12 hours) than that of the phosphatases (acid and alkaline phosphatase, glucose-6-phosphatase). In the ileum, where the inflammatory reaction was less severe and not characterized by significant epithelial degeneration, only a slight decrease in enzyme activity occurred at the peak of the lesion.

In another sequential study of enzyme changes in the small intestinal mucosa after acute injury, Spiro and Pearse[3] found an early increase followed by a severe decrease and rapid recovery of enzyme activity of mouse duodenal mucosa following whole-body x-irradiation. With a smaller x-ray dose Jonek, Kósmider and Kaiser[4] found only an increase in activity in 2 of the 3 enzymes studied. Decreases in small intestine epithelial enzyme activity have been demonstrated in guinea pigs with enteritis due to a coccidium[9] and in rats with enteritis due to a nematode,[10] but the rate of recovery was not investigated. In the small intestinal lesion of chronic celiac disease the abnormal surface epithelium has depressed enzyme activity.[1,2,6,17,18] In contrast to the transitory lesions observed in animals, in celiac disease the brush border alkaline phosphatase activity has been found to be normal by most investigators.[1,2,6,18] Spiro and associates[1] observed some return of enzyme activity in patients with celiac disease following a gluten-free diet and

this seemed to be related to an increase in the height of villus epithelial cells. Samloff, Davis and Schenk[2] found an increase in adenosine triphosphatase activity 3 to 5 hours after the institution of a gluten-free diet, but activity of other enzymes remained low. In Whipple's disease the surface epithelium of jejunal villi exhibits little alteration in hematoxylin and eosin stained sections and also has normal enzyme activity.[17] In all of these studies there also appears to be a close correlation between alteration in small intestinal epithelial structure and decrease in enzyme activity.

The striking change in mucosal lipid distribution in the jejunal and ileal mucosa of monkeys given enterotoxin suggests that the abnormal jejunal epithelium at the height of the lesion was not able to absorb lipid. Although the ingestion of fat was not controlled, the disappearance of lipid from the jejunal mucosa only at the height of the lesion (2 to 8 hours) and the appearance of lipid in the ileal mucosa slightly later (4 to 12 hours) suggests that fat passed the jejunum without being absorbed only at the height of the lesion. Samloff and co-workers[2] found an abnormal pattern of lipid distribution in the jejunal mucosa in celiac disease. They described lipid droplets in the lamina propria in normal people and in treated celiac disease and lipid in the epithelium but not in the lamina propria in untreated celiac disease.

The gastric mucosa exhibited only slight enzyme change in spite of well developed inflammatory lesions in the fundus and antrum. The enzymatic variation in the stomach and jejunum may be due to the difference in the nature of the inflammatory reaction. In the gastric mucosa the principal reacting cell other than the leukocyte was the mucous cell, whereas, in the jejunum the absorptive cells of the villi were severely injured. Surface gastric mucous cells exhibited a slight reduction in enzymatic activity at the height of the lesion when these cells were depleted of mucus. Deep mucous cells were not well demonstrated by the enzyme techniques used. Parietal cells appeared uninjured in conventionally stained sections and did not exhibit enzyme alterations. At the height of the lesion the cytoplasm of chief cells was shrunken and this was associated with an increase in acid phosphatase activity. In contrast to the glandular epithelium of the stomach, those covering the jejunal villi were severely altered both morphologically and enzymatically. These findings support the concept that mucous epithelium protects the underlying gastric glands from noxious agents while the absorptive cells of the small intestine are situated on the surface and are more vulnerable to injury.

The normal distribution of oxidative enzymes and glucose-6-phosphatase in rhesus monkey gastric and small intestinal mucosae

generally corresponds to that in other species, but there are differences in respect to alkaline and acid phosphatase.[19-21] The rhesus monkey is the only species studied which normally has alkaline phosphatase activity in the muscularis mucosae and smooth muscle fibers of the lamina propria. Acid phosphatase has strong activity in gastric chief cells in the rhesus monkey, but in small laboratory animals the activity is strongest in parietal cells or absent from both types of cells.[21] There are conflicting reports as to whether activity is strongest in the chief or parietal cells of man.[19,21]

SUMMARY

Rhesus monkeys given 150 μg of purified staphylococcal enterotoxin B developed acute gastroenteritis. This was well developed at 2 hours, maximal at 4 to 8 hours and rapidly regressed 12 to 72 hours after enterotoxin administration. These features were compared with changes in enzyme activities (acid and alkaline phosphatase, glucose-6-phosphatase, DPNH and TPNH diaphorases, succinic dehydrogenase, glucose-6-phosphate dehydrogenase and the hexokinase system). In the gastric mucosa at the height of the lesion there was a slight reduction of enzyme activity in surface mucous epithelium and a slight increase in acid phosphatase activity in chief cells. No change was demonstrated in parietal cell enzymes.

In jejunal epithelium a rapid decrease and recovery of enzyme activity was noted. This correlated well with alterations in the structure of the epithelium. In the ileum, where epithelial alterations were not conspicuous, changes in enzyme activity were slight.

Lipid droplets disappeared from the jejunal mucosa and appeared in the ileal mucosa at the height of the lesion suggesting an alteration in the pattern of fat absorption.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Acid phosphatase activity, gastric fundus. a. Normal. Moderate activity appears in chief and surface mucous cells. b. Four hours after enterotoxin. Increased activity is manifest in chief cells, decreased activity in surface mucous cells, and a strongly positive reaction appears in macrophages in the lamina propria. Both × 80.

FIG. 2. Alkaline phosphatase activity, jejunum. a. Normal. Strong activity appears in the brush border. b. Eight hours after enterotoxin there is no demonstrable activity. c. At 24 hours activity returns to near normal in the regenerating villi. All × 80.

FIG. 3. Acid phosphatase activity, jejunum. a. Normal. Strong activity is apparent in the apical cytoplasm, weak activity in the supranuclear region and strong activity in macrophages. b. Eight hours after enterotoxin. There is no activity in the apical cytoplasm, moderate activity in the supranuclear zone and variable activity in macrophages. Both × 315.
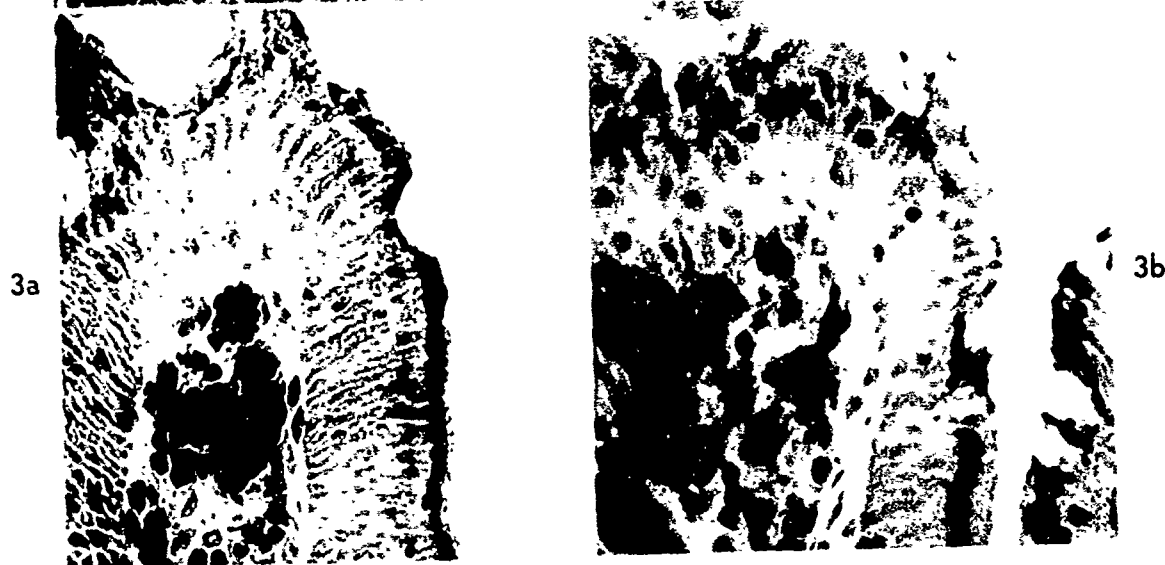
FIG. 4. Glucose-6-phosphatase activity, jejunum. a. Normal. Strong cytoplasmic activity is shown. b. Four hours after enterotoxin activity in the epithelium is weaker and more irregular. Both × 315.

FIG. 5. DPNH diaphorase activity, jejunum. a. Normal. Strong activity is apparent in villus epithelium and Paneth cells and weak activity in the upper crypt region. b. Four hours after enterotoxin activity over the surface of blunted villi is reduced. c. At 24 hours activity in surface epithelium has returned to near normal. All × 50.

FIG. 6. Succinic dehydrogenase activity, jejunum. a. Normal. There is strong activity in the basal cytoplasm and moderate activity in the apical region. b. Four hours after enterotoxin epithelium exhibits a severe decrease in activity. c. At 24 hours considerable return of activity has occurred. All × 330.

FIG. 7.   Glucose-6-phosphate dehydrogenase activity, jejunum.   a. Normal. Villus
epithelial cells exhibit strong activity.   b. Four hours after enterotoxin patchy
reduction of activity is evident in the surface epithelum.   c. At 24 hours ac-
tivity in the surface epithelium has returned to near normal. All × 50.

FIG. 8.   Hexokinase system activity, jejunum.   a. Normal. Surface epithelium ex-
hibits strong activity; activity in Paneth cells and macrophages is weak.   b.
Four hours after enterotoxin there is patchy reduction of surface epithelial
activity and an increase in the number of macrophages.   c. At 24 hours surface
activity has returned to near normal. × 50.

FIG. 9.   Oil red O stain, jejunum.   a. Normal. Small lipid droplets appear in the
epithelium and the lamina propria.   b. Four hours after enterotoxin no stain-
able lipid is demonstrable in the mucosa.   c. At 24 hours abundant lipid is evi-
dent in the mucosa. Lipid droplets appeared in the ileal mucosa 4 to 12 hours
after enterotoxin; this suggests that the lipid passed the abnormal jejunal
mucosa without being absorbed. All × 270.

7a   7b, c

8a   8b, c

9a   9b, c